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GENE FLOW IN MAINLAND AND INSULAR POPULATIONS OF *CRASSOSTREA* (MOLLUSCA)

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ABSTRACT

Insular and mainland populations of *Crassostrea* species were compared with respect to relative gene flow, levels of genetic variation, and population differentiation. The relative level of gene flow was reduced among Caribbean Island subpopulations as compared to mainland subpopulations along the Atlantic seaboard and Gulf of Mexico. However, this reduction in gene flow among island subpopulations has resulted in very little population differentiation between islands or between islands and mainland demes. This suggests that the existing level of insular gene flow may adequately override genetic drift, founder effect, and weak selection. The reduction in gene flow was, however, accompanied by a substantial drop in the average heterozygosity among island demes when compared to mainland populations.

INTRODUCTION

There are two opposing views concerning the significance of gene flow among natural populations. One proposes that gene flow is a conservative force which prevents population differentiation (Mayr, 1963; Dobzhansky, 1970; Stanley, 1979). The other holds that gene flow is ineffective and that populations which freely exchange genes may indeed differentiate as long as there exists different selection regimes within the distribution of a species (Ehrlich and Raven, 1969). Perhaps both views are partially correct. Recently an experimental method of studying gene flow among natural populations was developed. Slatkin (1981) has derived a method of estimating relative gene flow among natural populations by using the allele frequency data from protein gel-electrophoretic studies. He found that gene flow differs greatly among a wide range of species. Among populations of *Drosophila* species, the blue mussel, milkfish, and an annual plant, he found high levels of gene flow which he considers make such species nearly panmictic. For these species gene flow appears to be a conservative force, and a species may be considered as a single evolutionary unit as predicated by Mayr (1963), Dobzhansky (1970), and Stanley (1979). In contrast, Slatkin found salamanders to have relatively low levels of gene flow. Consequently, Slatkin's data gathered on the salamander species supported the views of Ehrlich and Raven (1969). Since the evidence supports two contrasting views on the importance of gene flow with reference to population differentiation, the two views were examined by comparing the effects of gene flow among contiguous shoreline populations with those of island populations. An outbreeding marine species with good larval dispersing capabilities was selected for study.

The study compares levels of gene flow among insular and contiguous mainland populations of marine pelecypod species of the genus *Crassostrea* which, like the blue mussel, *Mytilus edulis,* have long planktonic larval stages. There is reason to

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believe that a reduction in inter-island gene flow should occur. The constraints upon pelagic larval dispersion along continental coasts are few. The direction and velocity of water currents and the time of reproduction and length of pelagic life are the only limitations placed upon larval dispersion among mainland populations (Scheltema, 1977). In addition to these constraints, insular pelagic larvae will experience other limitations. Most pelagic larvae aggregate in the plankton and are transported in swarms. Consequently, if an island is small, larval swarms may pass by it without making contact (Thorson, 1961) thereby leading to sporadic inter-island population disjunction in gene flow. If differences in the level of gene flow can be found, what effect might this have on the levels of genetic variation and population differentiation within the species distribution?

The American oyster, *C. virginica,* is distributed along the eastern seaboard of North America and the Gulf of Mexico. South of the Yucatan peninsula and in the Caribbean Islands, the mangrove oyster, *C. rhizophorae,* replaces *C. virginica* (Stenzel, 1971). Both species have planktonic larval stages which last from two to three weeks providing ample opportunity for zygotic dispersion (Galtsoff, 1964). These species are considered to be very closely related and some workers consider them to be different varieties of the same species, *C. virginica* (Mattox, 1949; Galtsoff, 1964; Menzel, 1968; 1971). The two species have been hybridized in the laboratory with the F_1 generation showing an integration of both parental types (Menzel, 1973). Perhaps the major physiological difference between the two species is that *C. virginica* is considered a euhaline species while *C. rhizophorae* is a hyperhaline species (Gunter, 1951). The allozyme data is now available for both species so that Slatkin's (1981) method of estimating gene flow can be used to examine the relative level of gene flow among the insular and mainland populations.

MATERIALS AND METHODS

The mainland populations of *Crassostrea* species were from the Chesapeake Bay, the Atlantic seaboard, and the Gulf of Mexico (Buroker, 1983, 1984). Insular populations of *C. rhizophorae* consist of groups from the Dominican Republic, Guadeloupe Island, Puerto Rico (Hedgecock and Okazaki, 1984), and the Virgin Islands (Buroker *et al.,* 1979). The population groups were (1) Chesapeake Bay (10 collecting localities or subpopulations), (2) the Atlantic coast and Gulf of Mexico (18 subpopulations), and (3) Caribbean Islands (4 different islands). The collecting locales were considered subdivisions of a single population (area) to estimate the relative levels of gene flow within each of the three areas. Thirty homologous structural loci as detected by protein electrophoresis were compared among these populations (Appendix).

RESULTS

A list of the monomorphic and polymorphic structural loci for *Crassostrea* species from each of the three areas is provided (Appendix). The heterozygosities for the polymorphic loci and the overall average heterozygosities of the *Crassostrea* species from each area are also given (Appendix). Slatkin's (1981) procedure for estimating gene flow among natural populations was applied. Slatkin's conditional average frequency, $\bar{p}_{(i)}$, was computed for the allozyme data among the subpopulations from each of the three areas. For each area, conditional average frequencies were plotted against i/d, where i ranges from one to d, and d is the number of subpopulations (Fig. 1, Table I). Although this procedure is a qualitative rather than a quantitative technique, it can be used to determine relative differences in levels of gene flow

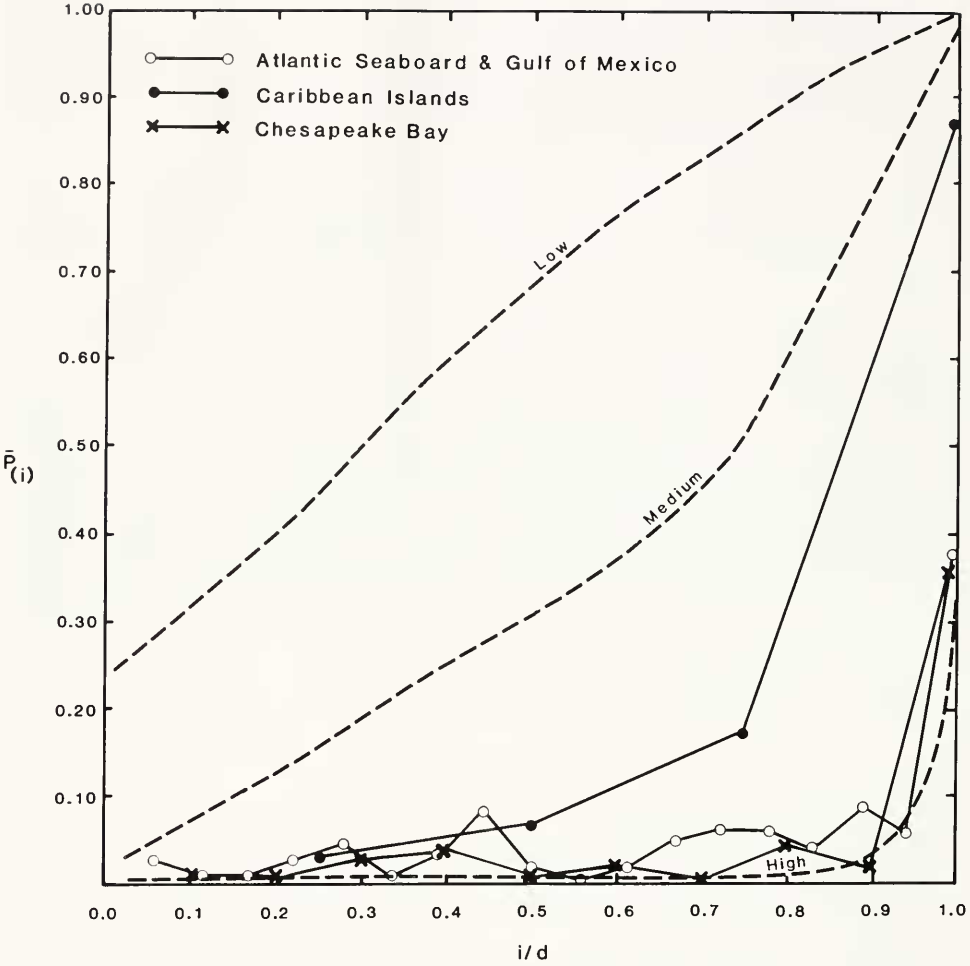

FIGURE 1. Plot of conditional average frequencies for different geographical regions of *Crassostrea* species. In each case $\bar{p}_{(i)}$ is plotted against i/d, where d is the number of sampling localities, to permit a comparison of mainland and insular populations. The dashed line depicts levels of gene flow among hypothetical populations.

between the three areas. Nei's (1975) coefficient of gene differentiation (G_{st}) for two $\bar{p}_{(i)}$ values was computed from each area (Table II). The coefficient can vary from zero (no gene differentiation) to one (complete gene differentiation) and provides information on the relative magnitude of gene differentiation among subdivisions within a population. It is used to compare the effects of gene flow between the Chesapeake Bay, the Atlantic Coast/Gulf of Mexico, and the Caribbean Island populations of *Crassostrea* species. The coefficient of gene differentiation can be related to Slatkin's (1981) procedure of estimating gene flow when the conditional average frequency of a given set of i/d intervals is used to determine the G_{st} value. In this instance, areas which have similar G_{st} values would presumably have similar levels of gene flow among their subpopulations while those with dissimilar G_{st} values would have a different level of gene flow among their subpopulations.

To compute the conditional average frequencies, $\bar{p}_{(i)}$, from allozyme data of three hypothetical species with low, medium, and high levels of gene flow, the plots of $\bar{p}_{(i)}$ with the appropriate i/d interval would appear as the broken line curves of Figure 1 (Slakin, 1981). In Figure 1, the conditional average frequencies are plotted against i/d from Table I as computed from the allozyme data of *Crassostrea* species from the Caribbean Islands (Buroker *et al.*, 1979; Hedgecock and Okazaki, 1984), the Chesapeake Bay (Buroker, 1984), and the Atlantic Coast and Gulf of Mexico (Buroker, 1983). Figure 1 shows little difference in the level of gene flow among collecting localities from the Chesapeake Bay as compared to those along the Atlantic seaboard

TABLE I

Geographical areas where Crassostrea *species were studied and background information for the data plotted in Figure 1*

	Caribbean Islands		Chesapeake Bay		Atlantic Coast & Gulf of Mexico	
i	i/d	$\bar{p}_{(i)}$	i/d	$\bar{p}_{(i)}$	i/d	$\bar{p}_{(i)}$
1	0.25	0.029 ± .020	0.10	0.008 ± .003	0.056	0.029 ± .060
2	0.50	0.065 ± .044	0.20	0.006 ± .001	0.111	0.010 ± .004
3	0.74	0.171 ± .125	0.30	0.028 ± .032	0.167	0.010 ± .004
4	1.00	0.870 ± .134	0.40	0.037 ± .043	0.222	0.043 ± .001
5			0.50	0.009 ± .001	0.278	0.045 ± .063
6			0.60	0.021 ± .027	0.333	0.010 ± .002
7			0.70	0.000	0.389	0.033 ± .034
8			0.80	0.043 ± .000	0.444	0.105 ± .163
9			0.90	0.019 ± .014	0.500	0.020 ± .003
10			1.00	0.362 ± .313	0.556	0.000
11					0.611	0.020 ± .016
12					0.667	0.049 ± .030
13					0.722	0.062 ± .074
14					0.778	0.061 ± .079
15					0.833	0.042 ± .028
16					0.889	0.092 ± .000
17					0.944	0.057 ± .000
18					1.000	0.381 ± .352
d		4		10		18
Polymorphic loci used*		6		16		21
Alleles		23		79		109
Range of sample size		4–54		36–93		27–138
Apparent level of gene flow		Med/High		High		High
Reference		Buroker *et al.*, 1979 Hedgecock and Okazaki, 1984		Buroker, 1984		Buroker, 1983

The data presented are the conditional average frequencies ($\bar{p}_{(i)}$ ± S.D.) for each interval (i/d), the number of sampling localities (d), the number of polymorphic loci studied, the number of alleles used, the range of sample size from each area and the apparent level of gene flow.

* Although other polymorphic loci are listed in the Appendix, not all were represented among every sampling locality; and therefore, could not be used in constructing Figure 1.

and Gulf of Mexico. They both have high levels of gene flow among subpopulations and are in agreement with Slatkin (1981) for *M. edulis* along the Atlantic seaboard. Among insular Caribbean subpopulations the plot of $\bar{p}_{(i)}$ against i/d falls between a medium and high level of gene flow (Fig. 1). This suggests that mainland populations of *Crassostrea* species have higher levels of gene flow than insular populations. To substantiate this result among the three study areas, the coefficient of gene differentiation (G_{st}) was computed for two corresponding conditional average frequencies of each area (Table II). If gene flow were less among island demes than mainland demes, a larger coefficient of gene differentiation among island demes than mainland communities would be expected. This was substantiated by the G_{st} results (Table II). Subpopulations in the Chesapeake Bay, the Atlantic coast, and Gulf of Mexico have approximately the same amount of gene differentiation (*i.e.*, very little). The lack of gene differentiation between these subpopulations results from similarly high levels of gene flow which overrides the other evolutionary forces of drift and weak selection. Table II also indicates that insular demes have greater gene differentiation than the other two areas. This suggests that less gene flow occurs between island demes than among contiguous mainland subpopulations.

The effect these factors have on levels of genetic variation within conspecific subpopulations proves interesting. Using average heterozygosity to estimate genetic variation, a great reduction in this estimate accompanies the reduction in gene flow. Individual heterozygosities, averaged over all loci, were approximately 22 and 24% for subpopulations in the Chesapeake Bay and along the Atlantic Coast/Gulf of Mexico, respectively. The same estimate averaged 12% within the Caribbean Island subpopulations (Appendix). This suggests that among these *Crassostrea* species a reduction in gene flow in conjunction with subpopulation inbreeding reduces the genetic variation necessary for evolutionary change. The lower average heterozygosities of insular populations of *C. rhizophorae* may not be species specific, since heterozygosities within mainland populations of *C. rhizophorae* (Hedgecock and Okazaki, 1984) and *C. virginica* (Buroker, 1983, 1984) are similar.

DISCUSSION

Low levels of genetic variation within Caribbean Island subpopulations of *C. rhizophorae*, as compared to mainland subpopulations of *Crassostrea*, may result from genotypic adaptations to oceanic islands. From this we might expect genetic differentiation between mainland and insular oysters. According to Hedgecock and Okazaki (1984) the amount of genetic differentiation is not great. They report the genetic similarity (Nei, 1978) among *C. rhizophorae* populations to be 0.97 between Santo Domingo in the Greater Antilles and Belize, Mexico and 0.95 between Santo

TABLE II

A summary of genetic parameters studied among three geographical areas of Crassostrea *species*

Area	d	i/d	$\bar{p}_{(i)}$	G_{st}
Chesapeake Bay	10	0.800	0.043	0.001
		1.000	0.362	0.002
Atlantic Coast & Gulf of Mexico	18	0.778	0.061	0.008
		1.000	0.381	0.004
Caribbean Islands	4	0.750	0.171	0.013
		1.000	0.870	0.041

The data presented are the number of collecting localities or subpopulations (d), the i/d intervals, the conditional average frequencies [$\bar{p}_{(i)}$] and the corresponding coefficient of gene differentiation (G_{st}).

Domingo and Trinidad. Even though gene flow is reduced among Caribbean Island subpopulations, it is still adequate in conjunction with the large insular demes to over-ride genetic drift, founder effects, and perhaps weak selection. A second explanation for low levels of genetic variation within the Caribbean Island subpopulations could result if the reduction in gene flow among subpopulations produces an imbalance between the occurrence of new alleles in the deme and the degree of inbreeding. If the insular deme size remains constant, the level of gene flow might effect the balance between the occurrence of new alleles and the degree of inbreeding within a subpopulation. For example, a high degree of gene flow in conjunction with a constant mutation rate would override the effects of inbreeding within a deme and the insular subpopulation would experience an influx of new alleles producing a relatively high level of genetic variation. In contrast, assuming the mutation rate remains the same, the situation could be reversed if gene flow were reduced. That is, inbreeding would eliminate alleles and erode away genetic variation within a deme. A third explanation for the low level of genetic variation among the Caribbean Island demes could result from the small sample size used in computing the average heterozygosities (Buroker *et al.,* 1979; Hedgecock and Okazaki, 1984). Smaller sample sizes are certainly biased against rare alleles. However, two independent studies (Buroker *et al.,* 1979; Hedgecock and Okazaki, 1984) provide standard errors with their estimates. These studies largely coincide and agree that average heterozygosity is significantly lower within insular demes when compared to mainland subpopulations. The final explanation concerns the insular deme size. With the mutation rate constant, we can assume that the frequency of neutral mutations maintained within a deme is directly related to its size (Crow and Kimura, 1970), and small demes should have lower levels of genetic variation then larger ones. This explanation can not be discounted since an accurate determination of the number of breeding individuals within a subpopulation is not available.

The smallest, most distant islands from the coastal mainland have the lowest levels of genetic variation among natural *C. rhizophorae* populations. For example, Puerto Rico, the Virgin Islands, and Guadeloupe Island have average heterozygosities of 9, 8, and 11%, respectively (Buroker *et al.,* 1979; Hedgecock and Okazaki, 1984). In contrast, the large Caribbean Island of the Dominican Republic, although very distant from coastal mainland populations, has an average heterozygosity of 15% for the endemic Santo Domingo oyster population. These data coincide with the *gene flow-variation* hypothesis described by Soulé (1971) in his study of the side-blotched lizard, *Uta stansburiana,* from the Gulf of California. Soulé assumes that populations throughout a species range are exposed to different selection intensities. Gene flow between these populations causes an exchange of endemic mutations which results in elevated genetic variation throughout the species range. The theory predicts that species differing in levels of gene flow many also differ in levels of genetic variation. For example, a species displaying a high level of gene flow as defined by Slatkin (1981) might maintain a larger number of rare alleles throughout its distribution than a species with a lower level of gene flow among populations. This is precisely what we see when we compare mainland populations of *Crassostrea* species (Buroker, 1983, 1984) with insular populations (Buroker *et al.,* 1979; Hedgecock and Okazaki, 1984). The work of Buroker *et al.,* (1979) and Hedgecock and Okazaki (1984) indicates that each island population has many rare alleles often endemic to that locality. Since these alleles are present in low frequency and levels of gene flow are reduced among insular as compared to mainland demes, these endemic rare alleles are not maintained throughout the Caribbean Island populations as they are among mainland populations of *Crassostrea* species. Consequently, a reduction in genetic variation within insular demes is experienced.

Soulé (1971) points out that natural populations on large islands often cannot be differentiated from mainland populations in their levels of variation. As Soulé shows, there is some asymptotic level of variation that is realized with increasing land area. Among the Caribbean Islands studied by Hedgecock and Okazaki (1984), the Dominican Republic (Santo Domingo) has by far the greatest land area (30,785 sq. km) followed by Puerto Rico (5528 sq. km), Guadeloupe Island (2044 sq. km), and the Virgin Islands (322 sq. km). Consequently, the Santo Domingo oyster subpopulation from the Dominican Republic, with its 15% average heterozygosity, is closer than any other Caribbean Island subpopulation studied to the apparent asymptotic level of 24% heterozygosity realized by mainland *Crassostrea* subpopulations.

In summary, I have presented evidence that relative gene flow is different between mainland and insular populations of *Crassostrea* species. In conjunction it has been observed that the reduction in gene flow accompanies lower levels of genetic variation within insular demes. Although gene flow was found to differ between insular and mainland *Crassostrea* populations, very little subpopulation differentiation (genetic divergence) was found with the decrease in gene flow. These results suggest that although gene exchange between demes is important in maintaining similarity in population structure they also indicate that the reduction in gene flow was not enough to cause genetic divergence among insular demes.

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APPENDIX

Protein loci used for genetic analysis

	Polymorphic loci and their heterozygosity					
	Chesapeake Bay		Atlantic Coast Gulf of Mexico		Caribbean Islands	
Protein locus	d	H	d	H	d	H
Aminopeptidase-1	10	0.765 ± .010	18	0.727 ± .008	1	0.333 ± .045
Acid phosphatase-3	7	0.697 ± .014	18	0.616 ± .009	1	0.479 ± .051
Adenylate kinase-1	10	0.614 ± .012	18	0.432 ± .009	3	0.259 ± .030
Aspartate aminotransferase-1	10	0.000	18	0.028 ± .003	4	0.000
Aspartate aminotransferase-2	10	0.388 ± .012	15	0.465 ± .011	4	0.088 ± .017
Aldolase	10	0.000	18	0.012 ± .002	1	0.000
Esterase-1	10	0.809 ± .009	18	0.627 ± .009	1	0.000
Glyceraldehyde 3-phosphate dehydrogenase	10	0.000	18	0.007 ± .001	4	0.000
Isocitrate dehydrogenase-1	10	0.045 ± .005	18	0.053 ± .004	1	0.056 ± .022
Isocitrate dehydrogenase-2	10	0.018 ± .003	18	0.038 ± .003	1	0.000
Lecine aminopeptidase-1	10	0.574 ± .012	18	0.608 ± .009	4	0.449 ± .031
Lecine aminopeptidase-2	10	0.357 ± .011	18	0.426 ± .009	1	0.286 ± .046
Malate dehydrogenase-1	10	0.011 ± .002	18	0.008 ± .002	4	0.017 ± .008
Malate dehydrogenase-2	10	0.023 ± .004	18	0.024 ± .003	3	0.083 ± .018
Mannose phosphate isomerase-2	10	0.801 ± .010	18	0.607 ± .009	1	0.630 ± .046
Muscle protein-1	10	0.000	18	0.063 ± .004	3	0.000
6-Phosphogluconate dehydrogenase	10	0.177 ± .009	18	0.266 ± .008	2	0.043 ± .018
Phosphoglucose isomerase	10	0.457 ± .012	18	0.479 ± .009	4	0.167 ± .023
Phosphoglucomutase-1	10	0.542 ± .012	18	0.589 ± .009	4	0.392 ± .031
Phosphoglucomutase-2	10	0.230 ± .011	18	0.324 ± .009	2	0.171 ± .047
Sorbitol dehydrogenase	7	0.211 ± .013	15	0.215 ± .008	1	0.000
Xanthine dehydrogenase	10	0.00	18	0.000	4	0.061 ± .015
	Monomorphic loci					
Acid phosphatase-1	7		18		1	
Adenylate kinase-2	10		18		1	
Esterase-2	10		18		1	
Hexokinase-1	10		18		2	
Malic enzyme (NADP) soluble	10		18		1	
Muscle protein-2	10		18		3	
Tetrazolium oxidase-1	10		18		1	
Tetrazolium oxidase-2	10		18		1	
Average heterozygosity (H_s)		0.224 ± .054		0.240 ± .055		0.117 ± .042

d is the number of subpopulations studied and H is the heterozygosity per locus ± S.E.